**Amendment and Response**
Serial No.: 09/180,340
Confirmation No.: 6674
Filed: 20 August 1999
For: STABLE RECOMBINANT YEASTS FOR FERMENTING XYLOSE TO ETHANOL

## Remarks

The Office Action mailed 27 March 2003 has been received and reviewed. Claims 1, 14, 18, 23, 25, 27 and 30 having been amended, the pending claims are claims are 1-34. Reconsideration and withdrawal of the rejections are respectfully requested

The amendment of claims 1, 23, and 25 to delete the phrase "a yeast having" is made to clarify the scope of the claims. It is applicants' position that the amendment does not narrow the scope of the claims.

Claims 14, 18, and 27 are amended to correct typographical errors.

Claim 27 is further amended to add a comma after the number "25." It is applicants' position that the amendment does not narrow the scope of the claims.

Claim 30 is amended to delete "forming" and insert "producing." It is applicants' position that the amendment does not narrow the scope of the claims.

## Summary of Interview

The Examiner is thanked for the courtesies extended to the undersigned during the telephonic interview of June 18, 2003. During the interview, the Examiner's written remarks at paragraph 9, which begins at page 10 of the Action, were discussed. Specifically, the meaning of the sentence "[t]hus, the reference anticipates the claimed invention" was discussed. The Examiner stated that Ho et al. (WO 95/13362) does not anticipate the claimed invention. Accordingly, the sentence "[t]hus, the reference anticipates the claimed invention" is wrong. Further, the meaning of the phrase "the yeast of Ho et al. would be expected to have the same properties of the claimed yeast as they are identical" at page 11, lines 6-7, of the Action was discussed. According to the Examiner, this phrase was intended to indicate that the yeast disclosed by Ho et al. include Saccharomyces, and not to indicate that the yeast disclosed by Ho et al. and the claimed yeast are identical in other respects. Claims 1, 14, 18, 23, 25, 28-30, and 34 were also discussed. Regarding claims 1, 23, and 25, the Examiner asserted it was unclear if the claims were directed to a product, or a product-by-process. Claims 1, 23, and 25 have been

amended to indicate they are directed to a product. No agreement was reached regarding the other claims.

**The 35 U.S.C. §112, Second Paragraph, Rejection**

The Examiner rejected claims 13, 15, 16-17, 19-28, 30, 32 and 33 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. These rejections are respectfully traversed.

Claim 13 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Action asserts that "claim 13 is indefinite because the claim recites 'at least about 10 ribosomal DNA sites' and the range of 'at least' is narrower that the range of 'about' which goes outside the first range." The Action further states that "a broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired."

The Action supports this assertion by citing *Ex parte Wu*, 10 USPQ2d 2031 (Bd. Pat. App. & Inter., 1989). Applicants note, however, the decision in *Ex parte Wu*, like the other decisions cited in the Action, address the use of the term "such as" and other similar phrases that may raise a question as to whether the feature introduced by the language is merely exemplary or required. In *Ex parte Wu*, the court found no such doubt with the use of the term "optionally," likening its use to accepted claim language like "up to," "0 to ...%," and "not more than." Applicants submit that, like these accepted terms, no such indefiniteness exists with the claimed phrase "at least about 10 ribosomal DNA sites."

Further, the M.P.E.P. makes clear that the term "about" is acceptable claim language (see e.g., M.P.E.P. § 2173.05(b)(A), a range "between 25 to about 45%" was held to be clear in *Ex parte Eastwood*, 163 USPQ 316 (Bd. App. 1968)). The addition of the language "at

least" does not render the claim indefinite as it merely reflects a range of values having a lower limit defined by the phrase "about 10 ."

It is applicants' position that the language "at least about 10 ribosomal DNA sites" clearly defines a range having a definite but flexible lower limit. Thus, the language "at least about 10 ribosomal DNA sites" in claim 13 does not render the claim indefinite.

Claim 25, as well as claims 22 and 23, are asserted to be "indefinite for the recitation of 'substantially retaining its capacity for fermenting xylose to ethanol', how much is considered to be 'substantial retention' as the term is not defined in the specification." The Examiner has failed to provide any legal support for the rejection and the rejection is without basis. If the Examiner is stating that a bright-line test must be provided to define the use of a relative term in a claim, the courts have repeatedly held that this is not required by the law. See MPEP §2173.05(b), in particular subsection D. General guidelines are all that is required, and this specification provides them.

Claim 27 is asserted to be "indefinite because the claim does not clearly indicate that the claim is written for dependency upon claims 1, 22, 23, 24 etc. in the alternative or whether the claim simultaneously is dependent upon all listed claims." Claim 27 is amended to recite "claim 1, 22, 23, 24, 25, or 26, to . . . ." It is applicants' position that claim 27 is dependent upon the recited claims in the alternative.

In claim 28, the recitation of "a second section marker" is amended to recite "a second selection marker."

Claim 30 and the claims dependent thereon are asserted to be indefinite for the recitation of a "method for forming cells." Claim 30 has been amended to recite a "method for producing cells."

For at least the reasons discussed above, applicants request reconsideration and withdrawal of the rejection of claims 13, 15, 16-17, 19-28, 30, 32 and 33 under 35 U.S.C. §112, second paragraph.

**First 35 U.S.C. §103(a) Rejection**

The Examiner rejected claims 1-34 under 35 U.S.C. §103(a) as being unpatentable over Ho et al. (WO 95/13362) in view of Hallborn et al. (Canadian Patent Application No. 2,090,122). This rejection is respectfully traversed.

The burden is on the Examiner to establish a prima facie case of obviousness of the claimed invention. According to MPEP § 2143, three criteria must be met to establish a prima facie case of obviousness. First, there must be a suggestion or motivation, either in the documents themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the document or to combine document teachings. Second, there must be a reasonable expectation of success. Finally, the prior art document (or documents when combined) must teach or suggest all the claim limitations. It is respectfully submitted that the Examiner has failed to establish a prima facie case of obviousness over the cited documents.

Ho et al. disclose yeast transformed with plasmid constructs containing the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase cloned into pLSK15 (a low copy number plasmid) or pUCKm10 (a high copy number plasmid) (see Ho et al., p. 15, line 29-p. 16, line 31). As explained by Ho et al., these vectors contain a yeast 2 μm replicon and replicate autonomously in *S. cerevisiae* and other yeast (see Ho et al., p.16, lines 2-3, 25, and 28-29).

Hallborn et al. is directed to "recombinant yeast strains transformed with xylose reductase and/or xylitol dehydrogenase enzyme genes," and state that "[i]f both of these genes are transformed into a yeast strain, the resultant strain is capable of producing ethanol on xylose containing medium during fermentation" (abstract). The authors teach a process for co-expressing xylose reductase and xylitol dehydrogenase in a yeast strain, where the process includes, inter alia, constructing yeast vectors each carrying one of the DNA sequences coding for xylose reductase and xylitol dehydrogenase (Hallborn et al., page 11, lines 19-28). Hallborn et al. do not teach or suggest the introduction of a gene encoding xylulokinase, and state that "both *S. cerevisiae* and *Sch. pombe* have a functioning xylulokinase gene" (Hallborn et al., page 2, line 31).

Yeast vectors that can be used include a plasmid that replicates autonomously (see Hallborn et al. at, for instance, page 7, lines 10-19, and the paragraph spanning pages 10 and 11). This plasmid can be a multicopy or single copy vector. Alternatively, the genes can be integrated into the yeast genome, for instance, at a ribosomal RNA locus (see Hallborn et al. at, for instance, page 7, lines 21-34, page 11, lines 7-8, and example 5). As taught by Hallborn et al., integration of foreign DNA into the yeast genome includes introducing a DNA fragment into a yeast that is then integrated. An autonomously replicating plasmid is also introduced with the fragment, but the autonomously replicating plasmid does not integrate; it is included because it carries a suitable marker for transformation, and is later removed from the cells (see Hallborn et al. at page 7, lines 27-31, and page 17, lines 24-30.

The Action states at page 7 that "Hallborn et al. also teach . . . a method of transforming cells with replicative and integrative plasmids." Applicants disagree. Hallborn et al. do not teach or suggest the use of plasmids that are both replicative and integrative. This is clearly taught at page 7, lines 10-34, where Hallborn et al. distinguish between the use of plasmids capable of replicating autonomously (lines 10-19) and the use of a DNA fragment to integrate a gene into the yeast chromosome (lines 21-34).

Motivation to combine referenced teachings.

"The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references" (MPEP §706.02(j) (emphasis added)).

The applicants respectfully submit that the requisite motivation to combine Ho et al. with Hallborn et al. cannot be found in either Ho et al. or Hallborn et al. It is axiomatic that motivation to combine the two documents cannot be attributed to the combination itself, and the Action does not show the existence in either cited document of a motivation to combine the

disclosures to produce the claimed invention. Each reference is examined in the following paragraphs for the existence of the requisite motivation.

The applicants submit that a fair reading of Ho et al. would certainly not lead one of skill in the art to combine the teachings therein with the teachings of Hallborn et al. Combining Ho et al. with Hallborn et al. would result in modifying the vectors of Ho et al. to no longer include a gene encoding xylulokinase. As Ho et al. teach that a gene encoding xylulokinase is required, there is no motivation in Ho et al. for making such a modification.

Likewise, Hallborn et al. provide no motivation to combine the two cited documents. Combining Hallborn et al. with Ho et al. would result in the yeast of Hallborn et al. also containing an introduced gene encoding xylulokinase. However, Hallborn et al. teach that "both *S. cerevisiae* and *Sch. pombe* have a functioning xylulokinase gene" (Hallborn et al., page 2, line 31). Thus, there is no motivation in Hallborn et al. for making such a modification.

The Action has also not presented any evidence that knowledge generally available to one of ordinary skill in the art would have provided the requisite motivation to combine the cited references. The Action states that "one of skill in the art would have been motivated to combine the teachings of the references because Ho et al. disclose that ethanol is an ideal liquid fuel for automobiles and Hallborn et al. disclose a method to perform stable transformations over time" (Action, sentence bridging pages 7 and 8). It is respectfully submitted that this is not a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references.

The Action also states that "it would have been obvious to one of ordinary skill in the art to arrive at the claimed invention as a whole because Ho et al. and Hallborn et al. teach the fermentation of sugars to ethanol . . . using the same strain of yeast (Action at page 7, second paragraph). The Office is requested to note that "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (MPEP §2143.01 (emphasis added))

Teaching or suggesting each and every limitation of the claimed invention.

Applicants submit that even if the cited documents were combined, there would be no reasonable expectation of success. Moreover, to establish a prima facie case of obviousness, the combined teachings must teach or suggest each and every limitation of the claimed invention (MPEP § 2143). It is respectfully submitted that the combined teachings of the two cited documents do not teach or suggest each and every element of independent claims 14, 18, 28, 29, 30, and 34.

*Method claims (independent claims 14, 18, and 30).* The method claims recite, inter alia, "transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a first selection marker" (claim 14), "transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a selection marker" (claim 18), and "replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence and containing the exogenous DNA " (claim 30). None of the cited documents teach or suggest the methods of claims 14, 18, or 30 that include the use of a replicative and integrative plasmid containing an autonomous replicating sequence and exogenous DNA. Thus, the cited documents do not teach or suggest each and every element of claims 14, 18, and 30.

*Product (vector) claims (independent claims 28, 29, and 34).* None of the cited documents teach or suggest a "plasmid vector comprising a functional yeast autonomous replicating sequence and an exogenous DNA . . . the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell" (claim 28), a "plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA . . . the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol" (claim 29), or a "plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA . . . the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell" (claim 34). None of the cited documents teach or suggest a plasmid vector

containing a functional yeast autonomous replicating sequence for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell. Thus, the cited documents do not teach or suggest each and every element of claims 28, 29, and 34.

For at least the reasons set forth above, applicants submit that claims the pending claims are nonobvious over the cited documents. Accordingly, the applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-34 in view of the cited documents.

**Second 35 U.S.C. §103(a) Rejection**

The Examiner rejected claims 1-34 under 35 U.S.C. §103(a) as being unpatentable over Ho et al. (WO 95/13362) in view of Lopes et al. (*Yeast* 1996;*12*(5):467-477). This rejection is respectfully traversed.

Ho et al. is discussed above. Lopes et al. disclose the "pMIRY integrative vector system, based on targeted integration into the yeast rDNA locus," and investigate possible reasons for the observed reduced mitotic stability of the integrative vectors when they contain a foreign gene (Lopes et al., abstract). The authors note that "pMIRY-type vectors are only stably maintained in the rDNA cluster if their size is smaller than or at most equal to the size of the rDNA unit (9.1 kb)" (Lopes et al., page 473, col.2).

"The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." Ex parte Clapp, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985); MPEP §706.02(j) (emphasis added). The Action does not provide any suggestion of the desirability of doing what the inventors have done.

The applicants respectfully submit that the requisite motivation to combine Ho et al. with Lopes et al. cannot be found in either Ho et al. or Lopes et al. It is axiomatic that

motivation to combine the two documents cannot be attributed to the combination itself, and the Action does not show the existence in either cited document of a motivation to combine the disclosures to produce the claimed invention.

Lopes et al. disclose that "pMIRY-type vectors are only stably maintained in the rDNA cluster if their size is smaller than or at most equal to the size of the rDNA unit (9.1 kb)" (Lopes et al., page 473, col.2). The smallest pMIRY-type vector disclosed by Lopes et al. is pMIRY1, which has a size of 6.1 kb. The DNA fragment disclosed by Ho et al. that includes the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase is at least 4.9 kb in size.[1] The addition of the Ho et al. DNA fragment to the smallest pMIRY-type vector would result in a vector of 11 kb. This is greater than the size limit of 9.1 kb disclosed by Lopes et al., and thus will result in a vector having reduced mitotic stability. Thus, there is no motivation to combine the cited documents.

The Action has also not presented any evidence that knowledge generally available to one of ordinary skill in the art would have provided the requisite motivation to combine the cited references. Specifically, the statement that "[o]ne of ordinary skill in the art would be motivated to combine the teaching of both references because the method taught by Ho et al. introduces DNA into the same yeast taught by Lopes et al." (Action, page 10, first full paragraph) is not a convincing line of reasoning. Further, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. In re Mills, 16 USPQ2d 1430 (Fed Cir. 1990) (MPEP §2143.01). The prior art does not suggest the desirability of the combination.

Applicants submit that even if the cited documents were combined, there would be no reasonable expectation of success. Moreover, to establish a prima facie case of obviousness, the combined teachings must teach or suggest each and every limitation of the claimed invention

[1]Ho et al. disclose that the size of the translated region of the xylulokinase gene is 2.1 kb (see Example 3). Ho et al. also disclose at Example 2 that the size of the xylitol dehydrogenase and its associated promoter are 1.9 kb and 910 bp, respectively. This is a total of 4.9 kb, and does not include the xylose reductase gene.

(MPEP § 2143). It is respectfully submitted that the combined teachings of the two cited documents do not teach or suggest each and every element of independent claims 14, 18, 28, 29, 30, and 34.

For at least the reasons set forth above, applicants submit that claims the pending claims are nonobvious over the cited documents. Accordingly, the applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-34 in view of the cited documents.

**Amendment and Resp nse**
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**Summary**

It is rcspcctfully submitted that the pending claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Reprcsentatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
Ho et al.

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The undersigned hereby certifies that this paper is being transmitted by facsimile in accordance with 37 CFR §1.8(d) to the Patent and Trademark Office, addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, on this 27th day of June, 2003, at 2:60pm (Central Time).

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**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE**
**Serial No.: 09/180,340**
**Docket No.: 290.00330101**

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted.

**In the Claims**

For convenience, all pending claims are shown below.

1. **(Amended)** A yeast which ferments xylose to ethanol, comprising:

[a yeast having] genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast, said genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase.

2. The yeast of claim 1 which also ferments glucose to ethanol.

3. The yeast of claim 2 which is Saccharomyces.

4. The yeast of claim 3 wherein said sites are non-transcribed DNA sites.

5. The yeast of claim 1 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

6. The yeast of claim 5 wherein the promoters do not require xylose for induction.

7. The yeast of claim 3 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

8. The yeast of claim 4 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol, the promoters also not requiring xylose for induction.

9. The yeast of claim 6 wherein the xylose reductase and xylitol dehydrogenase genes are from natural yeast which ferment xylose to ethanol.

10. The yeast of claim 9 wherein the natural yeast are Candida Shehatae, Pichia stipitis or Pachysolen tannophilus.

11. The yeast of claim 9 wherein the xyulokinase gene is from a yeast or bacteria.

12. The yeast of claim 11 wherein the xyulokinase gene is from Candida Shehatae, Pichia stipitis, Pachysolen tannophilus, Saccharomyces cerevisiae, Schizosaccharomyces pombe, or Escherichia coli.

13. The yeast of claim 1 having said genes integrated at least about 10 ribosomal DNA sites of the yeast.

14. **(Twice Amended)** A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(a) transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a first selection marker; and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of the [progency] progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

15. The method of claim 14, wherein the plasmid DNA also includes a second selection marker for selecting cells which include the plasmid.

16. The method of claim 14 wherein the cells are yeast or eukaryotic cells, and wherein the method further includes the step of repeatedly replicating the progeny cells from step (b) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

17. The method of claim 16 wherein the cells are yeast cells and the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, which also serve as the first selection marker.

18. **(Twice Amended)** A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly [replicative] replicating the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

19. Yeast cells produced by the method of claim 18.

20. The yeast cells of claim 19, wherein the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, and the yeast cells ferment xylose to ethanol.

21. The yeast cells of claim 20, wherein said genes are fused to non-glucose-inhibited promoters which do not require xylose for induction, and wherein the yeast cells ferment glucose and xylose simultaneously to ethanol.

22. Yeast cells according to claim 21 which substantially maintain their capacity to ferment xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

23. **(Amended)** A yeast which ferments xylose to ethanol, comprising:

[a yeast having] multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase fused to non-glucose inhibited promoters, the yeast fermenting glucose and xylose simultaneously to ethanol and substantially retaining its capacity for fermenting xylose to ethanol for at least 20 generations when cultured under non-selective conditions.

24. The yeast of claim 23, wherein said promoters do not require xylose for induction

25. **(Twice Amended)** A yeast which ferments xylose to ethanol, comprising:

[a yeast having] multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, the yeast fermenting xylose to ethanol and substantially

retaining its capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

26. The yeast of claim 25, wherein the promoters do not require xylose for induction

27. **(Twice Amended)** A method for fermenting xylose to ethanol, comprising fermenting a xylose-containing medium with a yeast of claim 1, 22, 23, 24, 25, or 26, to [product] produce ethanol.

28. A plasmid vector comprising a functional yeast autonomous replicating sequence and an exogenous DNA comprising a first selection marker, the exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further including a second section marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell.

29. A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of the target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol.

30. **(Twice Amended)** A method for [forming] producing cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence and containing the exogenous DNA to produce multiple generations of progeny cells while selecting for cells which

include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

31. The yeast of claim 1 wherein the yeast maintains xylose fermenting capability after culture in non-selective medium.

32. The method of claim 14 wherein the cells are yeast.

33. The method of claim 30 wherein the cells are yeast.

34. A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell.